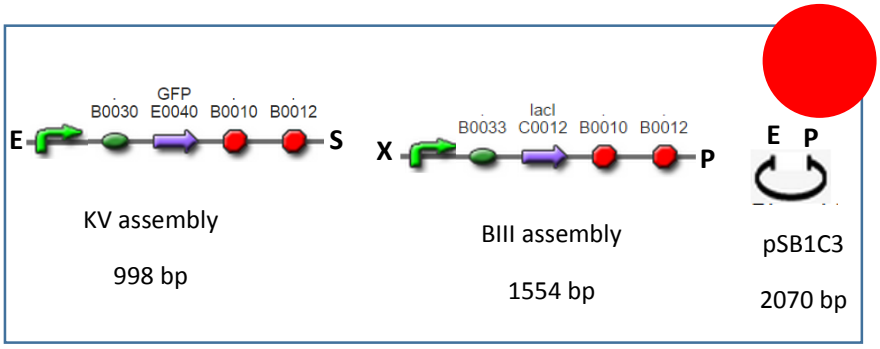
Assembly:

K XVI



1st Day

EXSP Digestion (see **Enzymatic Digestion Protocol**)

Parts	ng/ul	Volume to 2,5 ug (ul)	Buffer x10 (ul)	EcoRI (ul)	XbaI (ul)	SpeI (ul)	PstI (ul)	H_2O to 50ul (ul)
KV assembly	242	10,5	5	1	-	1	-	32,5
BIII assembly	156,9	16,0	5	-	1	-	1	27
pSB1C3	288,0	**3,5 ug** = 12 ul	5	1	-	-	1	31

Repeat this digestion only if you run out of stock

2nd Day

Gel Purification

- See **Kit Wizard SV gel and PCR clean up Promega Protocol**
- Quantify digestion products

Parts	ng/ul	260/280
KV assembly (ES)	10,1	1,97
BIII assembly (XP)	18,8	2,05
pSB1C3 (EP)	31,0	2,05

Obs: 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

Ligation (see **Ligation Protocol**)

Linear Plasmid 50 ng	2 ul	
Insert : Plasmid 3:1 (KV) ; 3:1 (BIII)	KV	BIII
	7 ul	6 ul
10x T4 DNA Buffer	1 ul	
T4 DNA ligase 1-5 u	0,5 ul	
H_20 to 10 ul	0,5 ul	

Obs: To determinate the amount of DNA necessary we used the following equation

$$Insert\ ng = plasmid\ ng \times \frac{insert\ bp}{plasmid\ bp} \times insert{:}\,plasmid\ ratio$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium
- Prepare glycerol 40%

3rd Day

Transformation (see **Transformation Protocol in *Escherichia coli* DH5-α**)

Organism: *E. coli* DH5-α

Selection: Cloranphenicol

4th Day

- Inoculate 3 – 4 colonies overnight in a 6 ml LB with the same antibiotic used in the transformation protocol.
- Incubate overnight at 275rpm/37°C.

5th Day

Miniprep

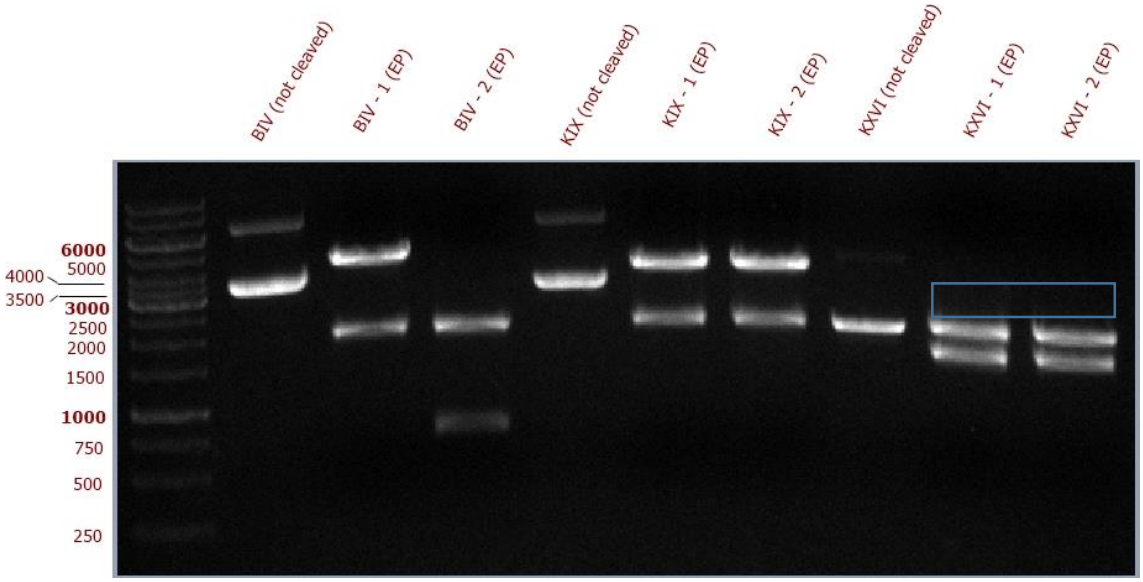
- Prepare **glycerol stock** of the clones (500ul glycerol 40% + 500ul inoculum).
- Extract plasmidial DNA (see **Alkaline Lyses or PureLink Invitrogen Protocol**)
- Run a preliminary electrophoresis gel.
- Quantify DNA samples.

Assembly Confirmation

- EP Digestion (see **Enzymatic Digestion Protocol**)

Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	EcoRI (ul)	PstI (ul)	H_2O to 10ul (ul)
KXVI -1	1	1	0,5	0,5	7
KXVI - 2	1	1	0,5	0,5	7

- Incubate for 2 hours at 37°C.
- Prepare samples for DNA sequencing.
- Run an electrophoresis analysis of the EP digestion



Size expected	Size in gel
2452 bp	~ 1500 bp

This assembly did not work. Analyzing the size of the insert in gel, we could say that the plasmid only incorporated the BIII assembly somehow. Because there was no more time we couldn't repeat the experiment unfortunately.